pore that the cell migrated through. Double strand breaks also increased and are consistent with subsequent cell death. The findings reveal a crucial role for the lamins in cell migration and survival, likely through DNA protection.

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The Kinetics of Nascent Protein Folding upon Release from the Ribosome

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Very little is known about the way proteins attain their native structure within the context of the living cell. In addition to the ribosome's well-established role in peptide bond formation, recent studies suggest that ribosomes play an important role in the early stages of protein folding in the cell and may be crucial for the production of folded bioactive proteins. Importantly, little is known about the impact of the mechanism of protein release from the ribosome on the attainment of a correctly folded conformation. Here, we present a kinetic study on the release time-course of fully synthesized ribosome-bound nascent proteins upon addition of the antibiotic puromycin. We focus these studies on the E. coli globin ApoHmpH. By time-resolved gel electrophoresis, we are able to follow puromycin's hydrolysis of the ester bond linking nascent polypeptides to the 3' end of tRNA. Steady-state fluorescence anisotropy allows us to follow the escape and folding of ApoHmpH from the ribosome. Finally, time decay fluorescence anisotropy analysis in the frequency domain complements the above techniques by providing insights into the local motions experienced by the nascent protein before and after release from the ribosome. Under experimental conditions where puromycin reacts at rates comparable to the naturally occurring release factors, we show that protein release from the ribosome is rate-limited by the C-terminal ester bond cleavage, and that escape from the ribosome and completion of folding occur quickly following this step. This result shows that the ribosomal context promotes a particularly ''temporally efficient'' folding upon nascent protein release. An important consequence of this phenomenon is the prevention of undesirable diffusion- and concentration-dependent phenomena such as aggregation.

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Diffusion Coefficient as a Function of Mass for Globular Biomolecules

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How well can the diffusion coefficient D of a globular biomolecule be predicted from its molecular mass MW? In ''Wanted: Scalable Tracers for Diffusion Measurements'' [J Phys Chem B, submitted], I propose that diffusion measurements in heterogeneous systems can be improved by the use of scalable tracers, in which the size is varied alone at constant shape, surface properties, diffusion mechanism, deformability, and other properties affecting diffusion. Before trying to design a de novo series of scalable globular proteins, it is appropriate to examine how scalable the commonly used de antiquo globular proteins are [ibid., supporting information]. The widely-used compilation of experimental diffusion coefficients by Tyn and Gusek [Biotech Bioeng 35 (1990) 327] was examined. This set – ranging from ribonuclease, 12640 Da, to tobacco mosaic virus, 50 MDa – was plotted as D versus log MW. The obviously linear species were removed, and values of D and MW for the outliers were examined. The plot yields a cloud of values of D versus log MW. In this plot, rigorously scalable tracers are expected to give a single smooth curve of D versus log MW, and the extent of the cloud represents scatter due to nonscalablity in the other properties, and to experimental error. Values of D from hydrodynamic calculations from various laboratories are remarkably consistent with the cloud. The cloud prediction is certainly good enough for semi-quantitative estimates or for designing single-particle tracking experiments. For a diffusion-controlled reaction in dilute solution, the prediction is close enough that the standard analysis of propagation of errors can be used. But arbitrary cloud proteins are not adequate for, say, measuring the percolation threshold of cytoplasm. The incomplete examination of the question here indicates what would be required for a complete examination.

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Developing a Nanocarrier for Targeted Delivery of Cardio-Protective Agents

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Background: Ischemic heart disease (IHD) is a leading cause of death worldwide. Pre-clinical drugs for IHD have largely failed in human clinical trials, highlighting the importance of developing vehicles to selectively deliver optimal therapeutic agents to the ischemic myocardium. Hydrophobins are fungal proteins that self-assemble into robust amphipathic monolayers that can encapsulate and solubilise hydrophobic drugs.
Aim: Develop the non-immunogenic hydrophobin RodA as a targeted-drug delivery vehicle.
Methods: Three recombinant RodA variants were prepared: RodA-MY, engineered to contain the peptide CSTSMLKAC that selectively binds to ischemic myocardium and RodA with or without a FITC-conjugate. RodA monomers and polymers were produced with and without the targeting sequence. All variants were tested for the ability of self-assemble into fibrillar (nanocarrier) structures. Monomeric and polymeric RodA variants were tested for cardiotoxicity using cardiomyocyte-like H9c2 cells. Targeting ability was tested using ischemia-challenged H9c2 cells and ischemic myocardium from a rat model of myocardial ischemia reperfusion.
Results and conclusions: Hydrophobins were well-tolerated by cultured H9c2 cells. Exposure of cells to both monomeric RodA or RodA polymers (50-100μg/mL) had no effect on cell viability or cell cycle profile. Immunocytochemistry demonstrated unaltered cell surface receptor distribution and activity as judged by binding and uptake of FITC-labelled transferrin. Consistent with previous data, the levels of the pro-inflammatory gene nFkB were unchanged suggesting that hydrophobins did not elicit inflammation in cardiac cells. Although, RodA-MY-nanocarrier has advantages over the native RodA-nanocarrier in targeting both normoxic and injured H9C2 cells, pilot work has revealed that RodA-MY-nanocarrier preferentially binds ex vivo to ischemic rat myocardium. Together the data indicate that hydrophobin-based polymers may act as drug-delivery vehicles for myocardial ischemic pathologies. **Future work:** studying the vehicles targeted ability of passing the vasculature barriers.

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The Nonrandom Nature of Weak Interactions between Proteins and Bystander Macromolecules in Cellular Environments

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Biochemical studies have mainly focused on specific interactions between ''reactant'' molecules, even though these interactions occur in cellular milieus crowded with ''bystander'' macromolecules. There is now growing experimental evidence that weak interactions of reactant proteins with macromolecular crowders may regulate biochemical processes [Miklos and Zhou, PLoS ONE 8, e74969 (2013)]. Computationally, our recent development of the FFT-based method for Modeling Atomistic Protein-crowder interactions (FMAP) has made it feasible to investigate the nature of such weak interactions [Qin and Zhou, JCTC 10, 2824 (2014)]. In FMAP, protein-crowder interactions, both hard-core and soft, are represented as correlations functions and evaluated via FFT, leading to the chemical potential of the protein in a crowded solution. Here we applied FMAP to three proteins whose interactions with crowders were subject to recent experimental studies. While the chemical potential comes from averaging all possible protein-crowder arrangements, we found that in each of the three cases a few ''hot'' regions on the protein surface make dominant contributions. Specifically, (1) a mutation of Ser16 to Glu on the Pin1 WW domain significantly reduces the magnitude of its chemical potential in concentrated ovalbumin solutions; (2) the chemical potential of a concentrated human growth hormone solution is dominated by two ''hot'' regions; (3) the domain cleft of the bi-lobed maltose binding protein is a ''hot'' region for interacting with bovine serum albumin as a crowder. These results are generally in line with experimental observations, though quantitative agreement will require further parameterization of protein-crowder interactions. The picture emerging from the computational and experimental studies is that weak protein-crowder interactions are nonrandom such that crowders and ligands may compete for the same sites for interacting with protein receptors, thus blurring the divide between specific and nonspecific interactions.

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A Computational Model for E. coli Cytoplasm: Diffusion and Hydrodynamics

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The dynamics of proteins is essential for the quantification of various cellular processes like rates of enzymatic reactions, signal transduction and protein

association reactions. However, understanding the structure and dynamics of macromolecules in a cell is complicated by the highly crowded nature of the cell. It is likely that properties of macromolecules in cell may differ significantly to that measured in dilute solution. Diffusion plays important roles in many processes occurring inside the cell. The estimation of diffusion coefficient of macromolecules in a cell can be considered as a first step in understanding the complex nature of the heterogeneous environment of the cell.
In this current work we developed a computational model of E. coli cytoplasm and performed extensive Brownian dynamics simulation to calculate diffusivity of proteins. Our model differs from some of the previous models of E. coli cytoplasm in the following way; (1) The proteins modeled as flexible units by considering them as a collection of spheres. (2) hydrodynamic interaction (HI), which is essential to get accurate diffusion coefficient, was considered using a mean field approach.
The model predicts accurately the diffusion coefficient of Green Fluorescent Protein (GFP) in E.coli cell. We have found that HI is essential to get correct diffusion coefficient for this highly crowded system. The presence of anomalous diffusion has also been observed for short time (~1 micro sec), which was identified using fractional Brownian motion (FBM) analysis. It was found that repulsive interaction between different proteins is the main reason for the anomalous diffusion. To understand the anomalous diffusion observed in simulations, we also formulated a one dimensional random walk model in which successive steps are biased and correlated. This analytical model can explain some of the findings from our simulation.

Voltage-gated K Channels I

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Movements of the Kv2.1 and Kv6.4 S4 Segments in Heterotetrameric Kv2.1/Kv6.4 Channels
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The voltage-gated K^+ (Kv) channel subunit Kv6.4 does not form functional channels on its own but tetramerizes with Kv2.1 subunits into functional Kv2.1/Kv6.4 heterotetramers with a proposed 3:1 stoichiometry. Within these Kv2.1/Kv6.4 heterotetramers, Kv6.4 causes an approximately 40 mV hyperpolarizing shift in the voltage-dependence of inactivation as compared to Kv2.1 homotetramers without affecting the voltage-dependence of activation significantly. By comparing the gating current (IQ) recordings of homotetrameric Kv2.1 and heterotetrameric Kv2.1/Kv6.4 channels, we recently showed that a second component in the charge (Q) versus voltage (V) distribution appeared in heterotetramers. Since this component develops at more negative potentials than Kv2.1 homotetramers, these results suggest that the voltage sensor of Kv6.4 subunits move in a more negative voltage range than the remaining Kv2.1's voltage sensors. Using cysteine accessibility studies, we show here that the voltage dependence of the rates of MTSET modification at V335C in Kv6.4 correspond with the second component of the QV distribution of Kv2.1/Kv6.4 heterotetramers. Similarly, the voltage dependence of modification rates at V296C in Kv2.1 follow the QV distribution of Kv2.1 homotetramers. These results indicate that in functional Kv2.1/Kv6.4 heterotetramers voltage sensors from Kv6.4 subunits move at more negative potentials than voltage sensors belonging to Kv2.1 subunits. (Supported by a FWO postdoctoral fellowship and FWO travel grant to EB and the Intramural Section Program of the National Institute of Neurological Disorders and Stroke, National Institutes of Health to MH)

584-Pos Board B364
Ion Channels and Salt Bridges: Quantum Calculations Show Unusual Effects
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Calculations on voltage sensing domains (VSD) of Kv1.2 (pdb: 2A79/3Lut), with a mutation in which cysteine replaces an S4 arginine that forms a salt bridge with S2 or S3 aspartate or glutamate residues allow an alternate interpretation of the results of MTS mutation experiments, as the cavity resulting from the mutation could accommodate an MTS reagent. The position of protons in the voltage sensing domains (VSD) alters the conformation of the aromatic residues, thus affecting intracellular vs. extracellular access of MTS reagents to the cys in the mutant, rendering ambiguous the results of MTS experiments on R→C mutants. In further work on salt bridges, we have done quantum calculations (all DFT: B3LYP/6-311++G**) on a salt-bridge-like ''ring'', in which a carboxylic acid and guanidinium (as in arginine) are separated by two water molecules, each connecting a carboxyl oxygen and a guanidinium nitrogen. Another complete set of calculations with a third, non-ring, water, showed how a small perturbation makes a large difference. Calculations in which one water was displaced by 1Å and (separate calculation) by 3Å, away from its ring position, (without reoptimization) demonstrate the effect of displacing one ring water on the charges of atoms of the other water, and on several other atoms, as well as on the bond orders, suggesting the ring shows resonance. For all cases, Natural Bond Order (NBO) calculations were also done. Separate calculations performed with the acid and base neutral, and ionized, indicate conformations of the ring depend on water molecule relations to the acid/base pair, as well as their charge state. Such rings may exist in proteins, including the VSD of ion channels.

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State Dependent Photo-Crosslinking of the IKS Channel Complex Demonstrates Movement of KCNE1 at Pre-Opening Membrane Potentials
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The slow delayed rectifier current (IKS) is a key repolarizing potassium current in the cardiac action potential. IKS is composed of KCNQ1 which forms the tetrameric voltage gated pore subunit and KCNE1, a single transmembrane domain accessory subunit, proposed to reside in the channel's exterior cleft. KCNE1 imposes a dramatic regulation on KCNQ1, significantly delaying opening compared to the unchaperoned channel. Here, we have investigated the dynamics of this interaction using the UV-crosslinking unnatural amino acid, p-benzoyl-L-phenyl alanine (pBpa). pBpa was genetically incorporated into KCNE1 at residue F57 in the transmembrane domain using the amber stop codon (TAG) suppression system. Characterization of the pBpa-incorporated channel complex revealed a 9 mV left shift in V0.5 of activation compared to wild type. To evaluate the channel's activation pathway, cells were held for 2s at a range of non-activating potentials (−110 - −30 mV) followed by a 4s activation step to +60 mV. Increasing the holding potential progressively reduced activation time confirming multiple closed-states. Crosslinking was induced for each non-activating potential by repeatedly applying a 300 ms flash of UV light at the end of the 2s hold followed by a 4s activation step to +60 mV. Analysis of the change in peak current vs. cumulative UV-exposure revealed a rapid decrease compared to wild type channels indicating the permanent trapping of closed channels. The greatest rates of crosslinking were found at the most hyperpolarized holding potentials but no significant change in rate was observed above −70 mV, indicating that KCNE1 has moved outside the pBpa crosslinking radius as the channels progress through the activation pathway. This initial movement of KCNE1 suggests that inhibition of KCNQ1 occurs in a closed-state closer to the open-state in the activation pathway.

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Testing the Hydration Status of the Shaker-K Channel Voltage Sensor Domain with Sugars
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Voltage gated K-channels are formed by two well defined domains: the Pore Domain (PD), which is responsible for the K+ ions conduction process, and the Voltage Sensor Domain (VSD), which sense the transmembrane potential due to the presence of several charged moieties. VSD moves upon activation such that ~4 net positive charges translocate across the membrane. The molecular details of such movement have been subject of intense controversy. We asked if part of the charges are hydrated at the resting and activated states, and if they change their hydration status during voltage activation. We measured the gating currents of a constitutively closed Shaker-V478W in macro patches of Xenopus oocytes in the presence of internal, external, or symmetric 2M Sucrose to reduce the water availability for eventual VSD hydration. Our results are consistent with the idea that some charged residues are hydrated when exposed to the cytosol at resting, dehydrate before translocation and rehydrate externally in the activated conformation. These suggest that water plays an important role stabilizing charged moieties in both, the resting and active conformation, revealing a novel role for water in the voltage sensing process.
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